

PATENT
Attorney Docket No.: 061537-0036

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: ARMEDARIZ BORUNDA *et al.* Confirmation No.: 4513
Application No.: 10/724,292 Group Art Unit: 1632
Filed: December 1, 2003 Examiner: Shin Lin Chen
For: RECOMBINANT ADENOVIRAL VECTORS AND THEIR UTILIZATION
IN THE TREATMENT OF VARIOUS
TYPES OF HEPATIC, RENAL AND
PULMONARY FIBROSIS AND
HYPERTROPHIC SCARS Attorney Docket No.: 061537-0036

DECLARATION UNDER 37 C.F.R. § 1.131

We, the inventors of the above identified application declare as follows:

1. We are the named co-inventors of the above-referenced U.S. Patent Application Number 10/724, 292 ("the '292 application"). We understand that the '292 application claims priority to U.S. Patent Application 10/098,359, filed March 18, 2002 and PCT Patent Application Number PCT/MX00/00035, filed September 14, 2000 and claims the benefit of Mexican Patent Application Number MX 998515, filed September 17, 1999. We also understand that the pending claims of the '292 application are directed pharmaceutical compositions comprising a therapeutically effective amount of unitary doses of viral particles of recombinant adenoviral vectors.

2. We have read and understand the Office Action dated April 7, 2006 and the Advisory Action, dated September 26, 2006. We understand that Hattori *et al.* (Human Gene Therapy, Vol. 10, No. 2, pp. 215-222) was published on January 20, 1999, and Jaffe *et al.* (Experimental Lung Research, Vol. 25, No. 3, pp. 199-215) was published in April-May 1999 and that these references have been cited as prior art against the pending claims. We understand that the submission of the certified copy of Mexican Application Number 998515 overcame the rejection of claims 22 and 28-35 under 35

U.S.C. § 102(b) and that based on a telephone conference with attorney for Applicants, Dean L. Fanelli, the Examiner considers pending claims 22 and 28-35 to be anticipated by the Hattori and Jaffe references under 35 U.S.C. § 102(a).

3. We conceived of the invention as claimed in pending claims 22 and 28-32 prior to the January 20, 1999 publication date of Hattori *et al.* (*i.e.*, the earliest relevant priority date). The prior conception of the subject matter of pending claim 22 and 28-32 is evidenced by the notebook pages dated March 31, 1998; April 7, 1998; April 18, 1998; April 23, 1998, April 24, 1998; April 27, 1998; April 28, 1998; and May 7, 1998, which reflects the work of the above-identified co-inventors disclosed in this application. The notebook pages are attached as Exhibit A and a certified translation is attached as Exhibit B. The claimed subject matter was therefore invented prior to the publication date of the Jaffe and Hattori references.


5. We further declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

Respectfully submitted,

Date: Nov 16, 2006

By: 
Juan Armendariz Borunda

Date: Nov 15, 2006

By: 
Estuardo Aguilar Cordova

20 de marzo de 1991

Ligan 5 circuitos de adenovirus con el gen de β -galactosidasa (traidos por el Dr. Estuardo Aguilar)

- Derivados de Ad5.
- Concentración = 1×10^{13} partículas virales (pv)/ml.
- Adenovirus 01496 AB galA.
- Número de circuitos = 5 (200044).

También llegaron dos plásmidos:

- pCMV-CAT (0.5 μ g/ μ l).
- pPCK- β gal (1.9 μ g/ μ l).

En ratones de 20 gr. se les administra los adenovirus por la vena de la cola (50-100 μ l), en diluciones de 10^7 - 10^{10} pv/ml. 48 hrs. después se sacrifican y hace la determinación de la actividad de β -gal en el hígado. La dosis letal en ratones es de 10^{12} pv/ml.

Dilución del Adenovirus

Buffer de dilución:

- Tris 10 mM pH 8.
 $g = (0.01 M) (0.25 L) (121.14) = 0.303 g$
- $MgCl_2 \cdot 6H_2O$ 2 mM.
 $g = (0.002 M) (0.25 L) (203.31) = 0.102 g$
- 4% de sacarosa
 $g = (4) (250) / 100 = 10 g$

31 de marzo de 1978

Transducción in Vitro de Macrófagos peritoneales de Raton.

Obtención de Macrófagos peritoneales de Raton.

El viernes 27 de marzo se les inyecta a cada ratón 1 ml de fagicolito de sodio al 3% por vía intraperitoneal (I.P.), para provocar irritación y la consecuente migración de macrófagos al peritoneo.

Después de 96 hrs. (3 d.) se sacrifica al ratón y se obtienen los macrófagos peritoneales como se indica a continuación:

- 1.- Desnucación del ratón, por dislocación cervical.
- 2.- Inyectar x vía I. P. 1 ó 2 ml de PBS estéril y frío, se le da un ligero masaje peritoneal.
- 3.- Hacer una pequeña incisión en el peritoneo y recolectar con una pipeta Pasteur estéril el PBS. Pasar el PBS a tubos de plástico fríos (en hielo).
- 4.- Centrifugar por 10 min. a 3500 r.p.m. a 4°C.
- 5.- Lavar dos veces el precipitado de células con PBS estéril frío (10 ml. cada lavado).
- 6.- Resuspender el precipitado con solución de Hanks (5-10 ml).
- 7.- Colocar los macrófagos en cajas de cultivo por un periodo de 2 hrs. a 37°C.
- 8.- Lavar las cajas con PBS a 37°C estéril.
- 9.- Adicionar RPMI-1640 a las cajas de cultivo y mantener las cajas a 37°C.

Martes 1 abril 1998

PRIMERA TRANSDUCCION IN VIVO.

Utilizamos 5 ratas hembras (200-250 grs.),
4 para administrarles adenovirus con el gen
lac-Z que codifica para la proteína β -galactosi-
dasa y 1 control sin adenovirus.

Les inyectamos 0.5 ml de una solución de ade-
novirus a cada rata por vía I.V. la vena -
elegida fue la vena iliaca. La relación de parti-
culas virales (pv) que le inyectamos a cada rata
es la siguiente:

Rata 1 1×10^{10} pv/ml
" 2 1×10^7 pv/ml
" 3 1×10^8 pv/ml
" 4 1×10^7 pv/ml
" 5 sin adenovirus

Se les va a dejar 48 con el adenovirus y
luego se les sacrificara para obtener órganos.

ADMINISTRACIÓN ENDOVENOSA DEL VECTOR
ADENOVIRAL (VENA ILIACA).

- 1.- Coloque a la rata en una cámara de
anestesia con una gasa empapada de
éter. Después de la anestesia, coloque
al animal en decúbito dorsal y sujé-
tele a la cama para cirugía.
- 2.- Lave con agua la extremidad inferior
a la que se le va a realizar la cirugía
y desinfectela con alcohol al 75%.

Sábado 18 de abril de 1998

Observamos los tejidos y no se percibió ninguna clase de tinción en algún corte de hígado de las 4 ratas, por lo que dejamos los tejidos en la suspensión de X-gal todo el fin de semana.

Lunes 20 de abril de 1998

RESULTADOS DE LA TRANSDUCCION IN VIVO (DOSIS RESPUESTA)

RATON No.	CORTES LAMINARES DE LOS SIGUIENTES ORGANOS		
	HIGADO	RIÑON	CEREBRO
1	-----	+++	-----
2	-----	+++	-----
3	-----	++	-----
CONTROL (-)	-----	+	-----

+ = Intensidad de la coloración

- = Sin tinción

CONCLUSIONES Y DISCUSIONES

- El pH de la suspensión de X-gal era de 7.0 de acuerdo a las tiras reactivas para medir el pH (debe ser mayor a 8 para eliminar la actividad de β -gal endógena que pudieran presentar los tejidos).
- Pudo observarse actividad β -gal endógena en los cortes de los riñones, puesto que hasta el riñón control sin adenovirus presenta una ligera coloración azul.
- El adenovirus pudo migrar preferentemente hacia el riñón.
- Se debe repetir el ensayo pero con una dosis más alta

de adenovirus.

Incrementar el pH de la suspensión de X-gal.

Repetición Del Ensayo Dosis Respuesta.

Se les administro el Ad-Bgal a 6 ratas Wistar machos de aproximadamente 250 gr. con las siguientes dosis:

- | | | |
|------|---|--------------------------------|
| Rata | 1 | 5×10^{10} pv. totales |
| ✓ | 2 | 5×10^{10} pv. totales |
| ✓ | 3 | 1×10^{11} pv. totales |
| ✓ | 4 | 1×10^{11} pv. totales |
| ✓ | 5 | 5×10^{11} pv. totales |
| ✓ | 6 | Control sin adenovirus. |

El volumen final que se les administro por rata fue de 500 μ l. conteniendo cada una de las dosis mencionadas anteriormente.

Jueves 23 de abril de 1998

Se sacrificaron las 6 ratas y se obtuvieron cortes laminares de hígado, riñón y cerebro. Todos los tejidos los lavamos 2 veces con PBS pH 8.0, se fijaron en formal por 10 min. y les reactivamos el revelado con el X-gal. El reactivo de X-gal, se les dejo por 12 hrs.

Se ligaron 8 ratas hembra, la rata 2 presento un paro respiratorio durante la cirugía pero salió bien, la 5 presento sangrado de la capa intramural del duodeno la cual se pudo volver a ligar.



TRANSPERFECT
TRANSLATIONS



AFFIDAVIT OF ACCURACY

I, Jamie Engels, hereby certify that the following is, to the best of my knowledge and belief, a true and accurate translation from Spanish into English.

Jamie Engels
TransPerfect Translations
601 13th Street, NW
Suite 320 North
Washington, DC 20005

ATLANTA
BOSTON
BRUSSELS
CHICAGO
DALLAS
DENVER
FRANKFURT
GENEVA
HONG KONG
HOUSTON
LONDON
LOS ANGELES
MIAMI
MINNEAPOLIS
MONTREAL
MUNICH
NEW YORK
PARIS
PHILADELPHIA
RESEARCH
TRIANGLE PARK
SAN DIEGO
SAN FRANCISCO
SEATTLE
STOCKHOLM
TOKYO
WASHINGTON, DC

Sworn to before me this
20th day of December, 2006

Signature, Notary Public

Lisa Sherfinski
Notary Public, District of Columbia
My Commission Expires 01-01-2008

Stamp, Notary Public
District of Columbia

March 20, 1998

5 cryovials of adenovirus with the β -galactosidase gene arrived (brought by Dr. Estuardo Aguilar).

- Derived from Ad5
- Concentration = 1×10^{13} viral particles (VP)/mL.
- Adenovirus 01496 AB gal A.
- Number of cryovials = 5 (200 μ L each).

Two plasmids also arrived:

- pCMV-CAT (0.5 μ g/ μ L).
- pPCK- β gal (1.9 μ g/ μ L).

The adenovirus was administered to 20-gram mice through the tail vein (50-100 μ L), in dilutions of 10^7 – 10^{10} VP/mL. 48 hours later, they were killed, and the determination of B-gal activity in the liver was carried out. The lethal dose for mice is 10^{12} VP/mL.

Dilution of Adenovirus

Dilution buffer:

- Tris 10 mM pH 8
 $g = (0.01 \text{ M}) (0.25 \text{ L}) (121.14) = 0.303 \text{ g}$
- $\text{MgCl}_2 \cdot 6 \text{ H}_2\text{O}$ 2 mM.
 $g = (0.002 \text{ M}) (0.25 \text{ L}) (203.31) = 0.102 \text{ g}$
- 4% saccharose
 $g = (4) (250) / 100 = 10 \text{ g}$

March 31, 1998

Transduction in Vitro of Peritoneal Macrophages from Mice

Obtaining Peritoneal Macrophages from Mice:

On Friday, March 27, each mouse was injected with 7 ml of sodium thioglycolate at 3% via intraperitoneal (IP), to provoke irritation and the consequent migration of macrophages to the peritoneum.

After 96 hours (today), the mouse was killed, and the peritoneal macrophages were obtained as indicated below:

- 1.– Break the mouse's neck, by cervical dislocation.
- 2.– Inject 1 or 2 mL of sterile and cold PBS via IP. Give a light peritoneal massage.
- 3.– Make a small incision in the peritoneum, and collect the sterile PBS with a Pasteur pipette. Transfer the PBS to cold plastic tubes (on ice).
- 4.– Centrifuge for 10 min. at 3,500 rpm at 4 °C.
- 5.– Wash the cell precipitate twice with cold, sterile PBS (10 mL each washing).
- 6.– Dissolve the precipitate again with Hanks solution (5-10 mL).
- 7.– Place the macrophages in culture flasks for a period of 2 hours at 37 °C.
- 8.– Wash the flasks with sterile PBS at 37 °C.
- 9.– Add RPMI-1640 to the culture flasks and keep the flasks at 37 °C.

Wednesday, April 1, 1998

FIRST TRANSDUCTION IN VIVO.

We used 5 female rats (200-250 grams), 4 to be administered adenovirus with the lac Z gene which codes for the protein β -galactosidase, and 1 control without adenovirus.

We injected 0.5 ml of a solution of adenovirus intravenously into each rat. The vein chosen was the iliac vein. The ratio of viral particles (VP) which we injected into each rat is the following:

Rat 1	1×10^{10} VP/mL
“ 2	1×10^9 VP/ml
“ 3	1×10^8 VP/ml
“ 4	1×10^7 VP/ml
“ 5	no adenovirus

They are going to be left for 48 with the adenovirus and then they are going to be killed to obtain organs.

INTRAVENOUS ADMINISTRATION OF ADENOVIRAL VECTOR (ILIAC VEIN)

1.— Place the rat in an anesthesia chamber with ether-saturated gauze. After the anesthesia, place the animal in dorsal decubitus and bind it to the surgical bed.

2. Wash the lower limb upon which the surgery is going to be carried out with water and disinfect it with 75% alcohol.

Saturday, April 18, 1998

We viewed the tissues and did not observe coloration of any sort in any slice of the livers of the 4 rats, and therefore we left the tissues in X-gal suspension for the whole weekend.

Monday, April 20, 1998

RESULTS OF THE TRANSDUCTION IN VIVO (DOSE RESPONSE)

RAT No.	LAMINAR SLICES OF THE FOLLOWING ORGANS		
	LIVER	KIDNEY	BRAIN
1	-----	+++	-----
2	-----	+++	-----
3	-----	++	-----
CONTROL (-)	-----	+	-----

+ = Intensity of coloration

- = No coloration

CONCLUSIONS AND COMMENTS

- The pH of the X-Gal suspension was 7.0 according to the reagent strips for measuring pH. (They should be greater than 8 to eliminate endogenous activity of the β -gal which the tissues could present.)
- Endogenous β -gal activity could be observed in the kidney slices, given that even the control kidney without adenovirus presents a light blue coloration.
- The adenovirus was able to migrate preferentially to the kidney.
- The assay should be repeated but with a higher dose

of adenovirus.

Increase the pH of the X-gal suspension.

REPETITION OF THE DOSE RESPONSE ASSAY

Ad-βgal was administered to 6 male Wistar rats of approximately 250 g with the following doses:

Rat 1	5×10^{10}	total VP
" 2	5×10^{10}	total VP
" 3	1×10^{11}	total VP
" 4	1×10^{11}	total VP
" 5	5×10^{11}	total VP
" 6		Control without adenovirus

The final volume administered per rat was 500 μL containing each of the doses noted above.

Thursday, April 23, 1998

The 6 rats were killed and laminar slices of the liver, kidney, and brain were obtained. We washed all the tissues twice with PBS pH 8.0. They were fixed in formaldehyde for 10 min., and we did the developing with the X-gal. The X-gal reagent was left for 12 hours.

Eight female rats were tied. Rat 2 suffered a respiratory arrest during the surgery, but survived. Rat 5 bled from the intramural layer of the duodenum, which we were able to tie again.

Friday, April 24, 1998

Rat 5, which bled during surgery, dies.

RESULTS OF THE DOSE RESPONSE (REPETITION)

RAT No.	LAMINAR SLICES OF THE FOLLOWING ORGANS		
	LIVER	KIDNEY	BRAIN
1	- +	-----	-----
2	+	-----	-----
3	- +	-----	-----
4	++	-----	-----
5	++++	+	+
CONTROL (-)	-----	-----	-----

Monday, April 27, 1998

Rat 7 is found dead in the morning.

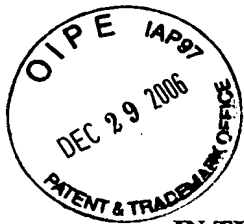
Tuesday, April 28, 1998

Adenovirus containing the lac-Z marker gene was administered to 2 rats whose bile ducts had been tied for 2 weeks. The amount of total viral particles per rat was 2.5×10^{11} in a total volume of 500 μ L. It was observed during administration that the full amount in the syringe entered without difficulty.

Friday, May 1, 1999

KILLING OF THE RATS TIED FOR 7 WEEKS

The two rats presented in apparently good health. They were killed and we obtained laminar slices of liver, kidney, and brain.



PATENT
ATTORNEY DOCKET NO.: 061537-0036

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: ARMEDARIZ BORUNDA <i>et al.</i>	Confirmation No.: 4513
Application No.: 10/724,292	Group Art Unit: 1632
Filed: December 1, 2003	Examiner: Shin Lin Chen
For: RECOMBINANT ADENOVIRAL VECTORS AND THEIR UTILIZATION IN THE TREATMENT OF VARIOUS TYPES OF HEPATIC, RENAL AND PULMONARY FIBROSIS AND HYPERTROPHIC SCARS	Attorney Docket No.: 061537-0036

SUPPLEMENTAL AMENDMENT UNDER 37 C.F.R. § 1.116

U.S. Patent and Trademark Office
Customer Window, Mail Stop AF
Randolph Building
Alexandria, VA 22314

Sir:

Further to the After Final Amendment submitted by the Applicants on August 7, 2006, Applicants have now received a certified copy of Mexican Application Number 998515, filed September 17, 1999 and include the original herewith. Applicants respectfully request entry of this Supplemental Amendment.

REMARKS

The Rejections Under 35 U.S.C. § 102(b)

Claims 22 and 23 were rejected on pages 6-7 of the office action, dated April 7, 2006, under 35 U.S.C. § 102(b) as allegedly being anticipated by Hattori *et al.*, January 20, 1999 (Human Gene Therapy, Vol. 10, no. 2, pp. 215-222) ("Hattori").

Claim 22 is also rejected on page 7 of the office action under 35 U.S.C. § 102(b) as allegedly being anticipated by Jaffe *et al.*, April-May 1999 (Experimental Lung Research, Vol. 25, No. 3, pp. 199-215) ("Jaffe").

Applicants respectfully submit that submission of the certified copy of Mexican application number 998515, filed September 17, 1999, perfects the priority claim, and therefore the rejection of claims 22 and 23 under 35 U.S.C. § 102(b) as anticipated by Hattori and the rejection of claim 23 under 35 U.S.C. § 102(b) as anticipated by Jaffe are no longer proper.

Applicants request that the rejections of claims 22 and 23 be reconsidered and withdrawn.

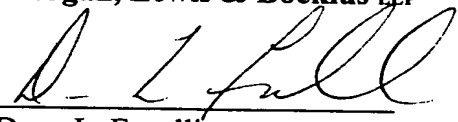
V. Conclusions

It is respectfully submitted that the rejections to the claims have been overcome. Should the Examiner disagree, Applicants respectfully request a telephonic or in-person interview with the undersigned attorney to discuss any remaining issues and to expedite the eventual allowance of the claims.

Except for issues payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-0310.

Dated: October 3, 2006
Morgan, Lewis & Bockius LLP
Customer No. **09629**
1111 Pennsylvania Avenue, N.W.
Washington, D.C. 20004
202-739-3000

Respectfully submitted,
Morgan, Lewis & Bockius LLP


Dean L. Fanelli
Registration No. 48,907